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(71) Applicant: CASE WESTERN RESERVE UNIVERSITY [US/US]; 2040 Adelbert Road, Cleveland, OH 44106 (US).

(72) Inventors: YOUNKIN, Steven, G. ; 2606 East Overlook Road, Cleveland Heights, OH 44106 (US). PALMERT, Mark, R. ; 2123 Lennox Road # 16, Cleveland Heights, OH 44106 (US).

(74) Agent: MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(54) Title: DIAGNOSTIC AND PROGNOSTIC METHODS BASED ON SOLUBLE DERIVATIVES OF THE BETA AMYLOID PROTEIN PRECURSOR

(57) Abstract

The present invention relates to methods for the prognosis, diagnosis, and staging of Alzheimer's Disease (AD), and to methods for monitoring response to therapy in a patient with AD. The methods of the invention involve the measurement of the levels in a sample of cerebrospinal fluid (CSF) of the ~25 kDa, ~105 kDa, and ~125 kDa soluble derivatives of the beta amyloid protein precursor (βAPP). In specific embodiments, detection of an increase in the percentage amount of the ~25 kDa protein and/or decrease in the percentage amount of the ~105 kDa derivative and/or high absolute levels of all three soluble βAPP derivatives, relative to healthy individuals, can be used to diagnose or prognose AD. The foregoing can also be used to diagnose Down's syndrome, to prognose disorders in Down's syndrome patients associated with amyloid deposition, and as an indication of neurologic aging. In other embodiments, determination of the percentage amounts of the ~25 kDa protein and/or the ~105 kDa protein relative to such amount present prior to therapy or in healthy individuals can be deemed a poor response to therapy of AD. The invention is also directed to the soluble ~25 kDa amino-terminal form of the βAPP.

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DIAGNOSTIC AND PROGNOSTIC METHODS BASED ON SOLUBLE DERIVATIVES OF THE BETA AMYLOID PROTEIN PRECURSOR

1. INTRODUCTION

The present invention relates to methods which can be used to diagnose, prognose, and stage Alzheimer's disease, and monitor response to therapy of Alzheimer's disease. Such methods involve the measurement of the levels in cerebrospinal fluid of the ~25 kDa, ~105 kDa, and ~125 kDa soluble derivatives of the beta amyloid precursor protein. The invention also provides for evaluation and monitoring of neurologic aging. The methods of the invention can also be used to diagnose Down's syndrome and prognose deleterious sequelae of amyloid deposition.

2. BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the aged and is a major cause of adult-onset dementia. It is characterized by cerebral depositions of amyloid. The principal proteinaceous component of the amyloid deposited in senile plaques and cerebral blood vessels in Alzheimer's disease is a 39-42 amino acid polypeptide (β amyloid protein, βAP) (Glenner G. G. and Wong, C. W., 1984, Biochem. Biphys. Res. Commun. 122:1131-1135; Masters et al., 1985, Proc. Natl. Acad. Sci. USA 82:4245-4249; Selkoe et al., 1986, J. Neurochem. 46:1820-1834) derived from a much larger membrane associated glycoprotein referred to as the β amyloid protein precursor (βAPP) (Goldgaber et al., 1987, Science 235:877-880; Kang et al., 1987, Nature 325:733-736; Robakis et al., 1987, Proc. Natl. Acad. Sci. USA, 84:4190-4194; Tanzi et al., 1987, Science 235:880-884; Kitaguchi et al., 1988, Nature 331:530-532; Ponte et al., 1988, Nature 331:525-527; Tanzi et al., 1988, Nature 331:528-530; Selkoe et al., 1988, Proc. Natl. Acad. Sci. USA 85:7341-7345; Palmert et al., 1988, Biochem. Biophys. Res. Commun.

156:432-437; Takio et al., 1989, Biochem. Biophys. Res. Commun. 160:1296-1303). The βAPP gene produces at least three mRNAs (Kitaguchi et al., 1988, Nature 331:530-532; Ponte et al., 1988, Nature 331:525-527; Tanzi et al., 1988, Nature 331:528-530) through alternative splicing of two exons (Kitaguchi et al., 1988, Nature 331:530-532). One of these exons encodes a 19-amino acid domain; the other encodes a 56-amino acid domain that is highly homologous to the Kunitz family of serine protease inhibitors. In each full-length precursor, the 39-to 42-residue βAP occurs as an internal sequence which extends from the extracellular region into the putative membrane-spanning domain (Kang et al., 1987, Nature 325:733-736; Dyrks et al., 1988, EMBO J. 7:949-947). The full-length forms of this precursor are truncated at their carboxyl-termini to produce ~125 and ~105 kDa (kilodalton) soluble derivatives (Schubert et al., 1988, Science 241:223-226; Schubert et al., 1989, Proc. Natl. Acad. Sci. USA 86:2066-2069; Weidemann et al., 1989, Cell 57:115-126; Podisny, M., et al., 1989, Soc. Neurosci. Abstr. 15(2):1379 (Abstr. 541.25); Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342; Palmert et al., 1989, J. Neuropathol. Exp. Neurol. 48:378 (Abstr. 231); Palmert et al., 1988, Biochem. Biophys. Res. Commun. 156:432-437)) that are readily detected in human cerebrospinal fluid (CSF) (Weidemann et al., 1989, Cell 57:115-126; Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342). Galasko et al. (1989, Soc. Neurosci. Abstr. 15(2):1376 (Abstr. 541.9) disclosed the detection of 88 kD and 100 kD proteins in human cerebrospinal fluid reactive with an antibody to the N-terminal region of βAPP. The ~125 kDa derivative, which appears to be the same protein as protease nexin-II (Oltersdorf et al., 1989, Nature 341:144-147 and Van Nostrand et al., 1989, Nature

341:546-549), contains the alternatively spliced (Kitaguchi et al., 1988, Nature 331:530-532) Kunitz protease inhibitor (KPI) domain, whereas the ~105 kDa form lacks this insert (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342). The identity of the ~105 kDa and ~125 kDa proteins in human CSF has been confirmed by purification and direct sequencing of their amino termini, both of which agreed with the sequence predicted from βAPP cDNAs (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342).

There are no definitive diagnostic markers for AD other than pathological changes that occur in brain, the most important of which are neurofibrillary tangles, neuritic plaques, and vascular amyloid. Diagnostic techniques less invasive than brain biopsy are greatly needed.

Alzheimer's type lesions containing amyloid protein are found in adult cases of Down's syndrome (Ball and Nuttall, 1981, Neuropathol. Appl. Neurobiol. 7:13).

3. SUMMARY OF THE INVENTION

The present invention relates to methods for the prognosis, diagnosis, and staging of AD. It further relates to methods for monitoring response to therapy in a patient with AD. The methods of the invention involve the measurement of the levels in a sample of cerebrospinal fluid (CSF) of the ~25 kDa, ~105 kDa, and ~125 kDa soluble derivatives of the βAPP. In specific embodiments, detection of an increase in the percentage amount of the ~25 kDa protein and/or decrease in the percentage amount of the ~105 kDa derivative and/or high absolute levels of all three soluble βAPP derivatives, relative to healthy individuals, can be used to diagnose or prognose AD. In another embodiment, such detection can be used to diagnose

Down's syndrome, and to prognose disorders in Down's syndrome patients associated with amyloid deposition. The foregoing can also be used as an indication of neurologic aging.

In other embodiments, determination of the percentage amounts of the ~25 kDa protein and/or the ~105 kDa protein can be used to stage AD, or to indicate the extent of mental dementia in a patient.

In embodiments directed toward monitoring therapy, an increase in the percentage amount of ~25 kDa protein and/or a decrease in the percentage amount of ~105 kDa protein relative to such amount present prior to therapy or in healthy individuals can be deemed a poor response to therapy.

The invention is also directed to the soluble ~25 kDa amino-terminal form of the βAPP.

4. DESCRIPTION OF THE FIGURES

FIG. 1. Amino-terminal sequence (single letter code) of the ~25 kDa soluble protein found in human CSF. X indicates that the signal was weak and did not permit an amino acid to be assigned. Note that the amino acid sequence predicted from βAPP cDNA (Kang et al., 1987, Nature 325:733-736) begins with a 17-residue signal sequence identified by Dyrks et al. (Dyrks et al., 1988, EMBO J. 7:949-957). Thus the amino-terminal residue of the mature protein is located at position 18 of the predicted sequence.

FIG. 2. Standard curves from a protein A experiment. These graphs show the relationship between bound ^{125}I protein A [shown as specific (total minus background) counts per minute (cpm)] and increasing amounts of βAPP. This is 1 of 4 such experiments which produced

standard curves for the ~125, ~105, and 25 kDa proteins with average r values of 0.9492 (n=3), 0.9614 (n=4), and 0.9409 (n=4), respectively (see Section 6.1 for details).

FIGURE 3. Derivatives that may be produced when full-length βAPP is cleaved generating soluble forms (adapted from Palmert et al., 1989, Biochem. Biophys. Res. Commun. 165:182-188). The solid bar in each schematic indicates the position of the βAP. The relative sizes of the membrane-associated and soluble forms (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342) and the observation that the soluble ~125 and ~105 kDa derivatives in CSF are labeled by antisera to the βAP (Palmert et al., 1989, Biochem. Biophys. Res. Commun. 165:182-188) indicates that cleavage normally occurs as shown in A or B. The cleavage shown in C may sometimes occur in normal individuals, and it could develop or be enhanced in AD.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods of prognosis and diagnosis of AD. It also relates to methods of staging AD and to monitoring response to therapy in a patient with AD. The invention is based upon the measurement in cerebrospinal fluid (CSF) of a patient of the levels of soluble derivatives of the βAPP, in particular, the approximately (~) 25 kDa, ~125 kDa, and ~105 kDa soluble forms. In another embodiment, the invention is directed to the soluble ~25 kDa amino-terminal form of the βAPP. The methods provided by the present invention also allow evaluation and monitoring of neurologic aging in an individual. The invention further provides methods for diagnosis of Down's syndrome.

The term "soluble" as used herein shall mean that which is "not cell-associated, not due to any artificial intervention," as opposed to the term "solubilized," which refers to that which is artificially released from a cell surface into solution, e.g., by detergent cell lysis.

As detailed in the Examples section, infra, we have quantitated the ~25 kDa, ~105 kDa, and ~125 kDa soluble N-terminal βAPP derivatives, and demonstrate (i) that in AD, there is a significant decrease in the relative amount of the ~105 kDa form and a corresponding significant increase in the relative amount of the ~25 kDa form (ii) that these changes correlate with the mental status of the AD patients, and (iii) that the same changes occur to a lesser extent in elderly as compared with young control patients.

5.1. MEASUREMENT OF SOLUBLE DERIVATIVES OF THE βAPP IN CEREBROSPINAL FLUID

The methods provided by the present invention involve the measurement of the levels of soluble derivatives of the βAPP in cerebrospinal fluid of a patient. Such soluble derivatives are selected from the group consisting of the ~25 kDa, ~105 kDa, and the ~125 kDa βAPP derivatives, which derivatives consist of a portion of the sequence of the βAPP starting with (as their amino-terminal residue) the amino acid at position 18 of the predicted βAPP sequence (see Kang, 1987, Nature 325:733-736).

According to the invention, the levels of one or more of the above-mentioned soluble derivatives are measured in CSF from a patient.

For the purpose of such measurement, CSF can be obtained by any procedures known in the art. For example, CSF can be obtained by lumbar puncture. Dialysis into

desired buffers and concentration before analysis can also be carried out. Measurement of the soluble βAPP derivatives can be by any techniques known in the art. In a preferred embodiment, an immunoblotting (Western blotting) procedure with appropriate antisera can be used to quantitate the soluble derivative(s) (see Examples sections, infra). For example, blots (e.g., nitrocellulose) of denaturing (e.g., sodium dodecyl sulfate-polyacrylamide) electrophoretic gels of separated soluble CSF proteins can be reacted with the appropriate antibody for detection and quantitation purposes. Such an appropriate antibody is one reactive with the soluble derivative being quantitated. For example, to quantitate all three ~25 kDa, ~105 kDa, and ~125 kDa derivatives with one antibody, antibodies which can be used include but are not limited to an antiserum against amino acids 45-62 in the βAPP sequence described by Kang et al., 1987, Nature 325:733-736) (anti-βAPP_{45-62}), and an antiserum to residues 18-35 of the βAPP. It is expected that antibodies recognizing an epitope within a ~25 kDa fragment of the βAPP starting at amino acid 18 should be suitable for such use. The antibody thus employed can be labeled, or it can be reacted with a labeled binding partner of the antibody (e.g. ^{125}I-labeled protein A) for detection and quantitation purposes. It is envisioned that other assays suitable for use will be known to those skilled in the art, including but not limited to assays employing one or more of the following techniques: chromatography (e.g.; ion exchange, immunoaffinity, immunoabsorption, and sizing chromatography such as high pressure liquid chromatography), centrifugation, electrophoretic procedures, differential solubility, competitive and non-competitive immunoassay systems using techniques such as

radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, precipitation reactions, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and immunoelectrophoresis assays, to name but a few; for example, immunoassay of HPLC fractions of CSF.

Antibodies against the soluble βAPP derivatives for use in immunodetection assays can be produced by methods known in the art. Such antibodies can be polyclonal or monoclonal.

For example, various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a given soluble βAPP derivative. For the production of antibody, various host animals can be immunized by injection with βAPP, or a fragment thereof, or synthetic protein or peptide corresponding to the foregoing, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species.

A monoclonal antibody can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256:495-497), and the human B cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) and others.

Antibody fragments which contain the idiotype (binding region) of the molecule can also be used, and can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

5.2. DIAGNOSIS, PROGNOSIS, AND MONITORING THERAPY OF AD

The quantitation of the soluble βAPP derivative(s) in CSF according to the present invention can be used for the diagnosis, staging and/or prognosis of AD. Such quantitation can also be used to monitor therapy of a patient with AD.

In a specific embodiment, detection of an increase in the percentage amount (% of total ~25 kDa + ~125 kDa + ~105 kDa soluble βAPP derivatives) of the ~25 kDa derivative and/or a decrease in the percentage amount of the ~105 kDa derivative, relative to healthy individuals, can be used as an indication of the presence or impending onset of AD, or in predicting the course of AD particularly when serial measurements are made. Increased percentage of the ~25 kDa protein and/or decreased percentage of ~105 kDa protein, and/or high absolute levels of all three soluble βAPP derivatives in non-demented individuals can be a significant risk factor such that a high percentage of the non-demented individuals who show such a profile eventually develop AD. The percentage amount of the ~25 kDa protein, and/or the percentage amount of the ~105 kDa protein, can be used to stage AD patients, and as an indication of the mental status of the AD patients (see, Examples sections, infra). Such percentage amounts can also be used to monitor therapeutic

strategies for AD, such as those aimed at reducing amyloid deposition. For example, an increase in the percentage amount of ~25 kDa derivative, and/or decrease in the percentage amount of ~105 kDa derivative relative to such amount present prior to therapy or in healthy individuals, can be deemed a poor response to therapy.

An increase in the percentage amount of the ~25 kDa protein, and/or a decrease in the percentage amount of the ~105 kDa protein, and/or an increase in the absolute amount(s) of one or more of the soluble βAPP derivatives, can also be used as an indication of neurologic aging. Thus, in particular embodiments, detection of such an increase for the ~25 kDa protein and/or decrease for the ~105 kDa protein, or increased absolute amounts, relative to young individuals (e.g., those individuals under age 60), can indicate neurologic aging.

In other specific embodiments of the invention, the ratios of the level(s) of one or more of the soluble βAPP derivative(s) to the level(s) of one or more different soluble βAPP derivatives can be calculated for use in the methods of the present invention. For example, the ratio of the amount of ~25 kDa protein to the amount of ~105 kDa protein in a sample of CSF can be determined for use in a method of diagnosis, prognosis, staging, or monitoring therapy, as provided by the present invention.

In yet another embodiment, the absolute level of one or more of the soluble βAPP derivatives can be determined for use in the methods of the present invention.

Kits for use in practicing the methods of the invention are also provided. Such kits comprise, in one or more containers, one or more antibodies directed against the soluble βAPP derivative(s) being quantitated.

5.3. DIAGNOSIS OF DOWN'S SYNDROME

The invention further provides methods for diagnosis of Down's syndrome. In specific embodiments, methods based on measurements of the soluble βAPP derivatives, including but not limited to detection of an increase in the percentage amount (% of total ~25 kDa and ~125 kDa and ~105 kDa soluble βAPP derivatives) in CSF of the ~25 kDa derivative and/or a decrease in the percentage amount in CSF of the ~105 kDa derivative, relative to healthy individuals, can be used as an indication of the presence of Down's syndrome or as an indication of the presence or impending onset in the Down's syndrome patient of disorders associated with amyloid deposition (e.g., mental dementia).

6. EXAMPLE: SOLUBLE DERIVATIVES IN THE β AMYLOID PROTEIN PRECURSOR IN CEREBROSPINAL FLUID: ALTERATIONS IN NORMAL AGING AND IN ALZHEIMER'S DISEASE

As described herein we isolated and sequenced a soluble ~25 kDa amino-terminal derivative of the β amyloid protein precursor (βAPP) that can be detected in human cerebrospinal fluid (CSF). In CSF samples from 24 Alzheimer's disease (AD) patients and 12 controls, we then quantitated this ~25 kDa form as well as the ~125 and ~105 kDa derivatives that we have previously identified (Palmert et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6338-6342). Our analysis shows (i) that, in AD, there is a significant decrease in the relative amount of the ~105 kDa form and a corresponding significant increase in the relative amount of the ~25 kDa form, (ii) that these changes correlate with the mental status of the AD patients, and (iii) that the same changes occur to a lesser exent in elderly as compared with young control patients. These observations indicate

that processing of the βAPP changes in normal individuals as they age and to a greater extent in those who develop AD.

6.1. METHODS

6.1.1. ANTISERUM

Production of antiserum to amino acids 45-62 (βAPP_{45-62}) in the βAPP sequence (Kang et al., 1987, Nature 325:733-736) has been described (Palmert et al., 1988, Biochem. Biophys. Res. Commun. 156:432-437), as has the characterization of this antiserum (Palmert et al., 1988, Biochem. Biophys. Res. Commun. 156:432-437; Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342). Briefly, a synthetic peptide corresponding to βAPP_{45-62} was conjugated to keyhole limpet hemocyanin for immunization of rabbits.

6.1.2. PURIFICATION AND SEQUENCING

The ~25 kDa protein was purified from CSF using ammonium sulfate fractionation, separation on a Mono-Q column (Pharmacia), and preparative SDS/PAGE as previously described for the ~125 and ~105 kDa proteins (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342). Briefly, the βAPP derivative was separated from the bulk (75%) of the CSF protein by fractionation at 62.5% of ammonium sulfate saturation (0.41 g/ml). The resulting pellet was resuspended in 20 mM sodium phosphate (monobasic) buffer (pH 6.8), desalted, and loaded onto a Mono Q column (Pharmacia). A linear 0 to 1 M NaCl gradient in 20 mM sodium phosphate (monobasic) buffer, pH 6.8, containing 0.05% Tween 20 was then applied to the column, and the ~25 kDa derivative (along with ~125 and ~105 kDa derivatives) was eluted in a well defined peak at ~60% (0.6 M NaCl) of the gradient. The fractions comprising this peak were pooled, desalted, concentrated, and subjected to

preparative SDS/PAGE on a 5-15% gradient gel. To concentrate the ~25 kDa derivative for sequencing, it was eluted from the preparative gel, electrophoresed in a single lane of another 5-15% SDS/PAGE gel, and transferred to Immobilon (Millipore Corp.). Sequence data was obtained using an Applied Biosystems 477A Sequenator.

6.1.3. PREPARATION AND QUANTITATION OF STANDARDS

Standards were prepared by dialyzing 25 ml of CSF from a control case (age = 74 yrs, postmortem interval = 5 hrs) against 20 mM sodium phosphate (monobasic, pH 6.8) and loading it onto a Mono-Q column. Bound proteins were eluted with a linear 0 to 1 M NaCl gradient in 20 mM sodium phosphate (monobasic, pH 6.8). The void volume and the portion of the gradient between 0 and 47.8%, which contains none of the three βAPP derivatives, were combined to produce a constant background of βAPP-free CSF protein. Those proteins that eluted between 47.8 and 100% of the gradient, including the βAPP derivatives, were then addedto the "background" protein in varying amounts to make standards that had a total protein content equal to that in our average CSF sample and that contained enough of the βAPP derivatives to span the range present in our CSF samples. The standards were exchanged twice into 1 mM dibasic/monobasic (1.6/1) phosphate buffer (pH 6.9) using Centricon-10 microconcentrators (Amicon) according to manufacturer's specifications. The concentrated standards were dried under vacuum and resuspended for SDS/PAGE on 5-15% gradient gels. Nitrocellulose blots were then blocked with 5% nonfat dried milk diluted in TBS (10 mM Tris-HCl, pH 8 and 150 mM NaCl); exposed overnight to anti-βAPP_{45-62} (Palmert et al., 1988, Biochem. Biophys. Res. Commun. 156:432-437; Palmert et al., 1989, Proc. Natl. Acad. Sci.

USA 86:6338-6342) diluted 1:100 in TBST (TBS (Tris-buffered saline) and 0.5% Tween-20); washed with TBST 4x for 15 minutes; exposed for 1 hour to 0.4 μCi/ml of [125]I protein A (ICN, specific activity 7.71 μCi/μg) diluted in TBST containing 5% nonfat dried milk; and washed with TBST 4x for 15 minutes. Autoradiograms were obtained so that labeled proteins could be visualized by superimposing the autoradiogram over the blot. Quantitation was achieved by excising each area of the nitrocellulose blot that contained the ~125, ~105, or major ~25 kDa protein. The size of the excised area corresponded to the typical band size and was uniform from experiment to experiment. We defined the specific cpm for each protein as the total counts in the excised area minus those present in an area of equal size cut from a lane which contained only the "background" CSF proteins.

6.1.4. INDIVIDUAL CSF SAMPLES

The clinical diagnosis of AD for the autopsied cases was established by a postmortem review of available medical records by an experienced clinician using standardized research criteria modified from the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1986, The Diagnostic and Statistical Manual of Mental Disorders. Third edition-revised. Washington). Pathological criteria for the diagnosis in these cases were similar to those suggested by a joint committee under the direction of the National Institute of Aging and recently validated (Tierney et al., 1988, Neurology 38:359-364). The diagnosis of AD in the living patients was established clinically by reviewing criteria suggested by the National Institute of Neurological and Communicative Disorders and Stroke with

the Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984, Neurology 34:939-944). This method is valid and reliable (Morris et al., 1988, Ann. Neurol. 24:17-22). All patients included were classified as probable AD. Three of the subjects from whom CSF was obtained during life have subsequently died and AD was confirmed at postmortem examination in all three. The clinical diagnosis of Parkinson's disease (PD) required the presence of at least 2 of the 4 major features of the idiopathic form of PD: resting tremor, stooped posture, shuffling gait, or muscular rigidity. No patients with secondary parkinsonism were included. Most of the living patients from whom CSF was obtained had received the "Mini-Mental Status Examination" (Folstein et al., 1975, J. Psychiat. Res. 12:189-198). This brief examination of mental function is highly reliable and valid; a score of 24 or below is compatible with a diagnosis of dementia. For the living patients, CSF was obtained by lumbar puncture with removal of 20 ml of fluid which was then aliquoted into 2 ml containers. Patients were at bed rest for several hours before and after the examination. All psychotropic medications were withdrawn prior to the lumbar puncture. CSF (240 µl) from each case was exchanged into 1 mM dibasic/monobasic (1.6/1) phosphate buffer (pH 6.9), concentrated, and analyzed as described above for the standards. Each experiment contained both AD and control samples, which were loaded into alternating lanes of each gel.

6.2. RESULTS

Using ammonium sulfate fractionation, fast protein liquid chromatography on a Mono Q column, and preparative SDS/PAGE (SDS-polyacrylamide gel

electrophoresis), we have isolated a ~25 kDa protein that is present in human CSF and that we previously showed (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342) to be specifically labeled by an antiserum against amino acids 45-62 in the βAPP (anti-βAPP_{45-62}). As shown in FIG. 1, we have sequenced 25 amino acids at the terminus of this ~25 kDa protein, 23 residues were detectable, and all 23 were identical to those predicted from the published cDNA sequence. The amino-terminal residue of the ~25 kDa protein, like that of the ~125 and ~105 kDa derivatives (Palmert et al., 1989, Proc. Natl. Acad. Sci. USA 86:6338-6342), is located at position 18 of the predicted sequence (Kang et al., 1987, Nature 325:733-736) after a 17-residue signal sequence (Dyrks et al., 1988, EMBO J. 7:949-957). On this basis we conclude that the ~25 kDa protein, like the ~125 and ~105 kDa proteins, is an amino-terminal derivative of the βAPP. It should be noted that the "~25 kDa derivative" consists of a major band and 1 or 2 minor bands that migrate at ~25 kDa on 5-15% SDS-polyacrylamide gels. All of these bands are specifically labeled by anti-βAPP_{45-62} and by an antiserum to residues 18-35 of the βAPP. Our sequence and the quantitative data shown below are from the major protein within this complex.

To assess the ~125, ~105, and ~25 kDa βAPP derivatives in control and AD CSF, we have used ^{125}I-labeled protein A to quantitate anti-βAPP_{45-62} immunoblots of CSF samples containing the three derivatives. Standard curves, which were generated by adding increasing amounts of the ~125, ~105, and ~25 kDa βAPP derivatives to a constant background of βAPP-free CSF protein, showed a satisfactory relationship between the added βAPP and the amount of ^{125}I-labeled protein A bound to immunoblots (Figure 2). Thus, this method is useful for assessing the

absolute amount of each βAPP derivative in CSF samples (measured as cpm ^{125}I-protein A bound to the immunolabeled protein in 240 μl of crude CSF). Moreover, it is particularly well suited for determining the relative amounts of the three βAPP derivatives because these proteins in each CSF sample are processed together and compared in a single gel lane.

The results of our assessment of CSF samples from 24 AD patients (10 samples were obtained at autopsy and 14 were obtained conventionally from living patients) and 12 non-demented controls (including 3 non-demented patients with Parkinson's disease, 3 with multiple sclerosis, 1 with recurrent encephalitis, 1 with acute psychosis, 1 with hypersomnia, and 3 with no neurological disease) are summarized in Tables I and II.

For Table I, to assess changes in the relative amounts of the 3 βAPP derivatives, the relative percentages were determined in each CSF sample and averaged to give the mean values tabulated here. The mean percentages calculated in this way give a true indication of the relative amounts of the βAPP derivatives present in the CSF of each patient group. These means cannot, on purely mathematical grounds, be determined reliably from the average absolute amounts shown in Table II. Statistical significance was verified using unpaired Student's t tests.

Samples from the living and autopsy AD patients both showed a reduction in the percentage of ~105 kDa form and an increase in the percentage of the ~25 kDa although the changes were slightly more pronounced in the samples obtained at autopsy.

TABLE I

RELATIVE AMOUNTS OF βAPP DERIVATIVES IN CONTROL AND AD CSF

Group	No.	Age	βAPP measured in CSF 25kDa	105kDa	125kDa
Young vs. Elderly Controls					
Non-demented controls (30-60 yrs)	7	45±4	13.9±3.1	70.4±2.8	15.6±2.4
Non-demented controls (62-79 yrs)	5	68±3	35.2±8.3 (p=0.02)	53.4±7.8 (p=0.04)	11.4±1.9 (p=0.23)
AD Cases by Mental Status					
Mini-mental:17-24	6	71±5	25.2±2.6	64.5±2.8	10.3±1.7
Mini-mental:11-16	4	74±3	42.5±8.1	45.0±6.5	12.5±2.1
Mini-mental:0	4	68±3	70.2±12.6	23.3±10.9	6.5±1.7
All AD and Control Cases					
Non-demented controls	12	55±4	22.8±4.9	63.3±4.3	13.8±1.7
All AD	24	73±2	47.3±4.9 (p=0.003)	42.2±4.2 (p=0.004)	10.5±1.2 (p=0.11)
Age-matched Cases					
Non-demented controls (55-79 yrs)	7	65±3	26.6±8.1	59.6±6.7	13.9±2.1
AD (52-73 yrs)	13	65±2	51.2±6.7 (P=0.04)	38.1±5.7 (p=0.03)	10.7±0.2 (p=0.33)

For Table II, the absolute amounts of the three βAPP derivatives in control and AD CSF are expressed in terms of cpm X 10^{-3} of ^{125}I-protein A bound to the immunolabeled protein and normalized for the amount of protein present in 240 µl of CSF. Because the protein content of the CSF samples showed little variation among the subgroups we examined, the same relationships were seen whether the absolute numbers were normalized for volume (240 µl) or protein content. Statistical significance was verified using unpaired Student's t tests (* denotes comparison between the least demented group of AD patients and the 12 controls). The protein content of the CSF samples showed little variation among the subgroups we examined. Thus, the same relationships were seen whether the absolute numbers were normalized for volume (240 µl) or protein content. The specific cpm values for each experiment have been corrected for the radioactive decay that occurred during the course of experimentation. N.B. If one calculates the relative amounts of each form from the average absolute data shown above, one does not obtain the values shown in Table I. These differences are not due to errors in compiling the data. They occur because on purely mathematical grounds the relative amounts calculated from the average absolute values do not reliably depict the average relative amounts that are present in the population. To accurately assess changes in the relative amounts of the βAPP derivatives, the relative percentages must be determined in each CSF sample and then averaged as we have done in Table I.

TABLE II

ABSOLUTE AMOUNTS OF βAPP DERIVATIVES IN CONTROL AND AD CSF

Group	No.	Age	βAPP measured in CSF 25kDa	105kDa	125kDa	Total
Young vs. Elderly Controls						
Non-demented controls (30-60 yrs)	7	45±4	1.5±0.6	6.2±1.6	1.3±0.4	9.0±2.5
Non-demented controls (62-79 yrs)	5	68±3	9.3±2.6 (p=0.006)	12.4±2.2 (p=0.04)	2.7±0.6 (p=0.06)	24.4±4.5 (p=0.009)
AD Cases by Mental Status						
Mini-mental:17-24	6	71±5	7.8±2.2*	25.0±8.3*	3.2±1.2*	36.6±11.4*
Mini-mental:11-16	4	74±3	3.6±1.0	3.6±0.5	1.0±0.1	8.2±1.0
Mini-mental:0	4	68±3	6.4±1.3 *(p=0.28)	3.7±2.7 *(p=0.02)	0.9±0.5 *(p=0.18)	11.0±3.9 *(p=0.03)
All AD and Control Cases						
Non-demented controls	12	55±4	4.8±1.6*	8.8±1.5*	1.9±0.4*	15.4±3.2*
All AD	24	73±2	6.1±0.7	9.6±2.8	1.8±0.4	17.7±3.7
Age-matched Cases						
Non-demented controls (55-79 yrs)	7	65±3	6.8±2.4	10.2±2.1	2.3±0.5	19.3±4.6
AD (52-73 yrs)	13	65±2	6.3±1.0	7.8±3.1	1.6±0.4	15.9±4.3

Comparison of CSF samples from the elderly controls (62-79 years, mean ± SE = 68 ± 3) with those from the young controls (30-60 years, mean = 45 ± 4) showed significant changes in both the relative and absolute amounts of the 3 βAPP derivatives. In the seven young controls, 14% of total βAPP was the ~25 kDa form, 70% was the ~105 kDa form, and 16% was the ~125 kDa form. In the five elderly controls, the relative amount of the ~25 kDa form was significantly higher at 35% (p~0.02), the relative amount of the ~105 kDa form was significantly lower at 53% (p=0.04), and the relative amount of the ~125 kDa was reduced non-significantly to 11% (p=0.23) (see Table I). The elderly controls also showed a more than 6-fold increase in the absolute amount of the ~25 kDa derivative (p=0.006), a 2-fold increase in both the ~105 kDa (p=0.04) and the ~125 kDa (p=0.06) forms, and a more than two-fold increase in total βAPP derivatives (p=0.009) (Table II).

The shift toward a higher percentage of the ~25 kDa form and a lower percentage of the ~105 kDa form that we observed in elderly controls occurred to an even greater extent in AD patients. In 24 AD patients as compared to the 12 controls, the relative amount of the ~25 kDa protein increased significantly from 23 to 47% (p=0.003), the relative amount of the ~105 kDa protein decreased significantly from 63 to 42% (p=0.004), and the relative amount of the ~125 kDa form decreased non-significantly from 14 to 11% (p=0.11) of the total βAPP (Table I). Since the AD patients were on average 18 years older than the controls, we compared an age matched subset that consisted of samples from the 7 oldest controls (55-79 years, mean = 65 ± 3) and the 13 youngest AD patients (52-73 years, mean = 65 ± 2). These age-matched AD cases also showed a significant increase in the percentage of the ~25 kDa protein (p=0.03), a significant

reduction in the percentage of the ~105 kDa form (p=0.04), and a non-significant reduction in the percentage of the ~125 kDa form (p-0.33) (Table I).

Of the 24 AD CSF samples analyzed, 10 were obtained at autopsy and 14 were obtained conventionally from living patients whose mental status was assessed. When the results from the 14 living AD patients were grouped according to the results of Mini-mental testing, a striking trend became apparent (see Table I). As dementia became more severe, the percentage of the ~25 kDa form rose progressively from 25% in patients with a Mini-mental score of 17-24, to 42% in patients with a score of 11-16, and 70% in patients with a score of 0. Conversely, the percentage of the ~105 kDa form decreased progresively from 65% in patients with a Mini-mental score of 17-24, to 45% in patients with a score of 11-16, and 23% in patients with a score of 0.

In the least demented group of AD patients, the percentages of the 3 forms were essentially the same as in the 12 non-demented controls. There was, however, a striking increase in the absolute amount of the ~105 kDa form (p=0.02) that, along with non-significant increases in the ~125 (p=0.18) and ~25 kDa (p=0.28) forms, resulted in a significant increase in total βAPP (p=0.03) in the least demented AD patients as compared to the 12 controls (see Table II). These changes, which were primarily due to extraordinarily high levels of βAPP derivatives in 3 of the 6 patients examined, could be due to an overall increase in βAPP production or to an increase in the rate at which soluble derivatives are produced from the full-length forms.

6.3. DISCUSSION

Two factors that contribute significantly to amyloid deposition in other amyloidoses are aberrant catabolism and increased levels of the various amyloidogenic precursors (Cohen, A.S., and Connors, L.H., 1987, J. Pathol. 151:110; Castano, E.M. and Fragione, B.F., 1988, 58:122-132 and Kisilevsky, R., 1988, 65:1805-1815). Our analysis of the βAPP derivatives in CSF indicates that both factors may contribute to amyloid deposition in AD. First, the percentage of the ~105 kDa form is decreased and the percentage of the ~25 kDa form is increased in normal aging and, to a greater extent, in AD, indicating that βAPP processing is altered in these conditions. Second, the absolute levels of soluble βAPP derivatives are increased in elderly control subjects and, to a greater extent, in the least demented AD patients.

If one considers young controls, elderly controls, slightly demented, moderately demented, and severely demented AD patients as a continuum, then our data show (i) a continuous increase in the relative amount of the ~25 kDa form (with overlap of the elderly control and slightly demented patients) (lines 1-5, Table I) and (ii) an increasing level of the soluble derivatives (particularly the ~105 kDa form) that peaks in the slightly demented group and then declines in the moderately and severely demented AD patients (lines 1-5, Table II). These changes may be due to (i) accelerated proteolysis of the large soluble βAPP derivatives (particularly the ~105 kDa KPI-free form) that leads to an increase in the relative amount of the ~25 kDa form and (ii) increased production of soluble derivatives due either to an overall increase in βAPP production or to an increase in the rate at which soluble forms are produced from full-length forms. Changes in the relative rates of these 2

opposing processes (production and degradation of βAPP derivatives) would readily account for the changes we observed in our comparison of young controls, elderly controls, and AD patients with dementia of increasing severity. Moreover, these changes could play an important role in amyloid deposition either by increasing the turnover of normal amyloidogenic intermediates or by generating aberrant, βAP-bearing forms from which amyloid could be generated.

The altered relative amounts of the ~25 and ~105 kDa derivatives in CSF correlate remarkably well with amyloid deposition in the sense that they change modestly in the elderly population where there is limited amyloid deposition, show more marked alteration in AD patients where amyloid deposition is pronounced, and change progressively as the severity of dementia increases in the AD population. The AD and age-matched control populations, though significantly different, were overlapping with respect to the absolute and relative levels of βAPP derivatives measured in CSF. These measurements may be useful (i) as part of a series of tests aimed at diagnosing AD, (ii) in predicting the course of AD particularly when serial measurements are made, and (iii) in monitoring therapeutic strategies aimed at reducing amyloid deposition. In addition, it is possible that an AD-like profile for these variables (increased % ~25 kDa and decreased % ~105 kDa, as was observed in the moderately and severely demented AD patients, or high absolute levels of βAPP, as was observed in the least demented AD patients) is a significant risk factor and that a high percentage of the non-demented individuals who show such a profile eventually develop AD.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

The present invention is not to be limited in scope by the embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A substantially purified protein consisting of an amino acid sequence identical to a portion of the beta amyloid precursor protein, which portion (a) has a molecular weight of approximately 25 kilodaltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis; and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is the leucine.

2. A method for diagnosing Alzheimer's disease in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased percentage amount of the soluble ~25 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the presence of Alzheimer's disease in the patient.

3. The method according to claim 2 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the presence of Alzheimer's disease in the patient.

4. A method for diagnosing Alzheimer's disease in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the presence of Alzheimer's disease in the patient.

5. A method for prognosing Alzheimer's disease in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble

~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased percentage amount of the soluble ~25 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the impending onset or progression of Alzheimer's disease in the patient.

6. The method according to claim 5 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the impending onset or progression of Alzheimer's disease in the patient.

7. A method for prognosing Alzheimer's disease in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25,

~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the impending onset or progression of Alzheimer's disease in the patient.

8. A method for staging Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which the percentage amount of the soluble ~25 kilodalton protein is indicative of the stage of Alzheimer's disease.

9. The method according to claim 8 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which the percentage amount of the soluble ~105 kilodalton protein is indicative of the stage of Alzheimer's disease.

10. A method for staging Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which the percentage amount of the soluble ~105 kilodalton protein is indicative of the stage of Alzheimer's disease.

11. A method for determining the extent of dementia in a patient with Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25,

~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which the percentage amount of the soluble ~25 kilodalton protein is indicative of the extent of dementia.

12. The method according to claim 11 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which the percentage amount of the soluble ~105 kilodalton protein is indicative of the extent of dementia.

13. A method for determining the extent of dementia in a patient with Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which the percentage amount of the soluble ~105 kilodalton protein is indicative of the extent of dementia.

14. A method for monitoring the effect of a therapeutic treatment on a patient with Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased percentage amount of the soluble ~25 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient prior to the treatment indicates a poor response to the therapeutic treatment.

15. The method according to claim 14 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage

amounts present in healthy individuals or in the patient prior to the treatment indicates a poor response to the therapeutic treatment.

16. A method for monitoring the effect of a therapeutic treatment on a patient with Alzheimer's disease comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient prior to the treatment indicates a poor response to the therapeutic treatment.

17. A method for diagnosing an early stage of or prognosing Alzheimer's disease in a patient comprising: measuring the total amount in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid

sequence identical to a portion of the beta amyloid precursor protein, and (b) comprise the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased total amount of the soluble ~25, ~105, and ~125 kilodalton proteins relative to those amounts present in healthy individuals of the patient's approximate age, indicates the presence of an early stage of or impending onset of Alzheimer's disease.

18. A method for the detection of neurologic aging in a patient comprising: measuring the total amount in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprise the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased total amount of the soluble ~25, ~105, and ~125 kilodalton proteins relative to those amounts present in young individuals indicates neurologic aging.

19. A method for the detection of neurologic aging in a patient comprising measuring the amount of a soluble protein in a sample of cerebrospinal fluid obtained from the

patient, in which the protein (a) has a molecular weight of approximately 25 kilodaltons as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; (b) consists of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (c) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly,

in which the amino-terminal residue is the leucine; and in which an elevated level of the soluble protein relative to those levels present in young individuals indicates neurologic aging.

20. A method for diagnosing Down's syndrome in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein, and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased percentage amount of the soluble ~25 kilodalton protein relative to those percentage amounts present in healthy individuals indicates the presence of Down's syndrome in the patient.

21. The method according to claim 20 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals indicates the presence of Down's syndrome in the patient.

22. A method for diagnosing Down's syndrome in a patient comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals indicates the presence of Down's syndrome in the patient.

23. A method for prognosing a disorder associated with amyloid deposition in a patient with Down's syndrome comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of a soluble ~25 kilodalton protein, a soluble ~105 kilodalton protein,

and a soluble ~125 kilodalton protein, and calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~25 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and in which an increased percentage amount of the soluble ~25 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the impending onset or progression of a disorder associated with amyloid deposition in the patient.

24. The method according to claim 23 which further comprises calculating the percentage amount, among the ~25, ~105, and ~125 kilodalton proteins, of the ~105 kilodalton protein, in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient in an earlier time indicates the impending onset or progression of a disorder associated with amyloid deposition in the patient.

25. A method for prognosing a disorder associated with amyloid deposition in a patient with Down's syndrome comprising: measuring the amounts in a sample of cerebrospinal fluid obtained from the patient, of an ~25 kilodalton soluble protein, an ~105 kilodalton soluble protein, and an ~125 kilodalton soluble protein, and

calculating therefrom the percentage amount among the ~25, ~105, and ~125 kilodalton proteins of the ~105 kilodalton protein, in which the ~25, ~105, and ~125 kilodalton proteins each (a) consist of an amino acid sequence identical to a portion of the beta amyloid precursor protein, and (b) comprises the following sequence at its amino-terminus:

leu-glu-val-pro-thr-asp-gly-asn-ala-gly

in which the amino-terminal residue is leucine; and in which the kilodalton molecular weights are as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and in which a decreased percentage amount of the soluble ~105 kilodalton protein relative to those percentage amounts present in healthy individuals or in the patient at an earlier time indicates the impending onset or progression of a disorder associated with amyloid deposition in the patient.

FIG. 1

-25 kDa PROTEIN: LEVPTDGNAGLLAEPQIAMFxGxLN

PREDICTED βAPP: LEVPTDGNAGLLAEPQIAMFCGRLN
18 42

FIG. 2A

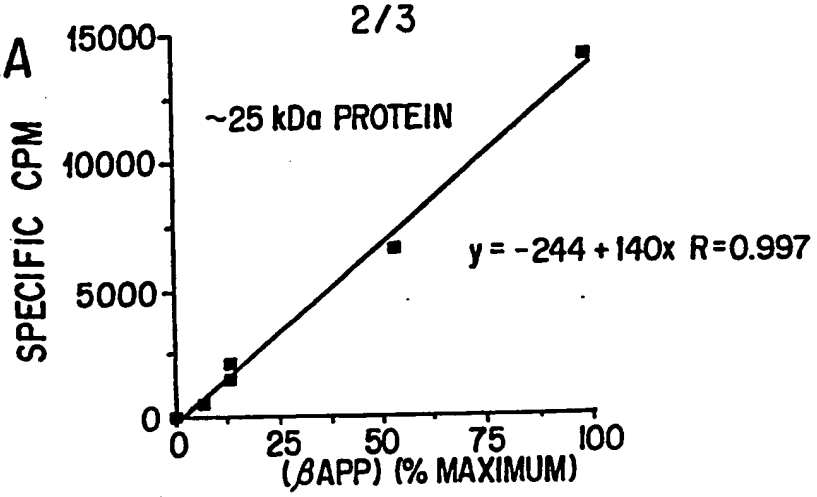


FIG. 2B

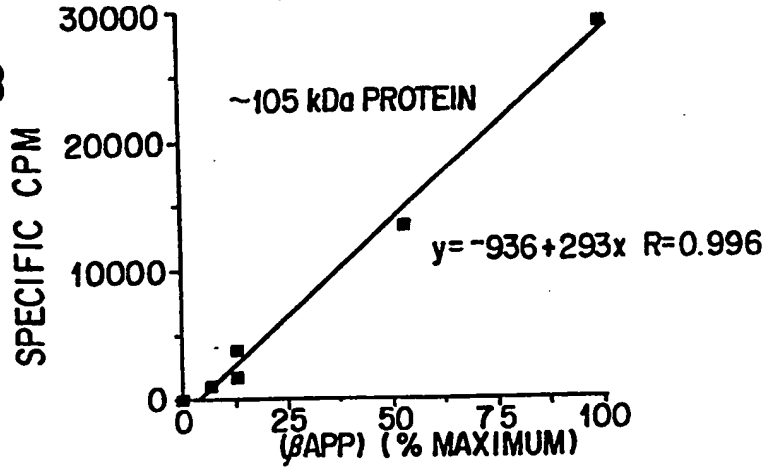
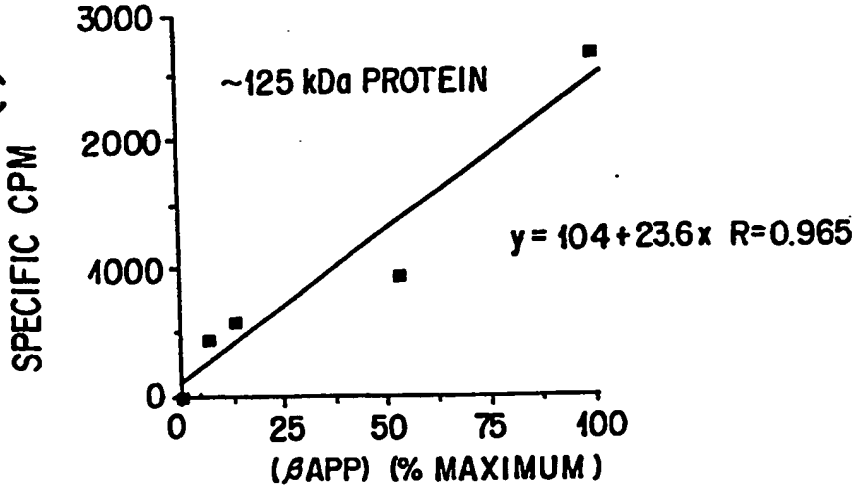


FIG. 2C



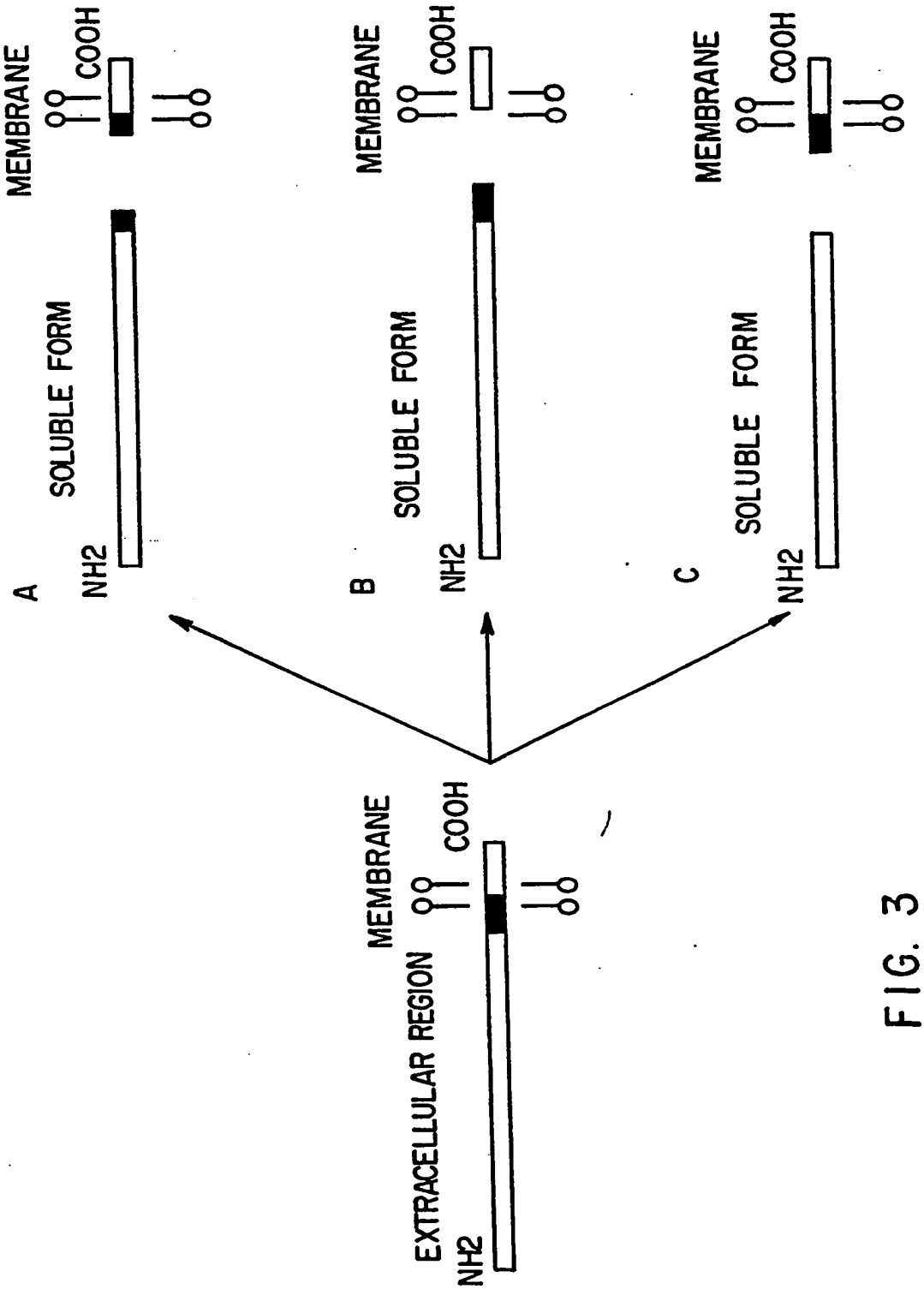

A
SOLUBLE FORM
NH2
MEMBRANE
COOH
B
SOLUBLE FORM
NH2
MEMBRANE
COOH
C
SOLUBLE FORM
NH2
MEMBRANE
COOH
EXTRACELLULAR REGION
NH2
MEMBRANE
COOH

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04607

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/00; C07K 7/00, 13/00
US CL: 436/86, 63; 530/324, 806; 930/10;

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US, CL.	436/86,63; 424/1.1; 435/6, 7.92; 530/324, 328, 806; 930/10

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS SEARCH (TERMS: ALZHEIMER? AND (CEREBRAL(w) SPINAL(W)FLUID OR(CSF))

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Journal Biological Chemistry, Vol. 262, No. 18, issued 1987, Van Nostrand et al., "Purification of Protease Nexin II From Human Fibroblasts", p. 8508-8514, see Table II, p. 8512.	1
X	NATURE, Vol. 341, Issued 12 October 1989 Van Nostrand et al, "Protease Nexin-II, a Potent anti-Chymotrysin, Shons Identity To Amyloid B-Protein Precursor," p.546-549, See Figure 1.	1
P,X	Neurology, Vol. 40, No. 7, Issued July 1990, Palmert et al., "Soluble Derivatives Of The Beta Amyloid Protein Precursor in Cerebrospinal Fluid: Alterations in Normal Aging and in Alzheimer's Disease," p. 1028-1034, See Abstract Only.	1-25

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 August 1991	23 OCT 1991
International Searching Authority	Signature of Authorized Officer
ISA/US	William Chan

Form PCT/ISA/210 (second sheet) (Rev.11-87)